SUPPLEMENTARY MATERIALS

Analyses of the oncogenic BRAF^{D594G} variant reveal a kinase-independent function of BRAF in activating MAPK signaling

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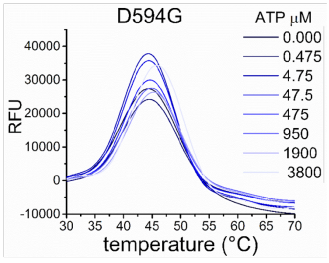
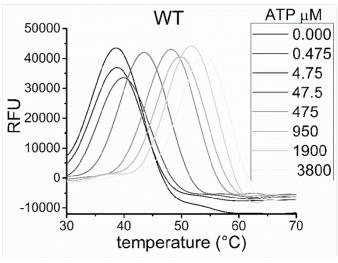
A



ATP µM	ΔT$_m$ °C
0.000	0.00
0.475	0.09
4.75	-0.03
47.5	0.03
475	0.28
950	0.53
1900	0.90
3800	1.47

B



ATP µM	ΔT$_m$ °C
0.000	0.00
0.475	0.12
4.75	1.34
47.5	4.86
475	9.54
950	11.16
1900	13.03
3800	15.40

Figure S1: BRAF^{D594G} cannot Bind ATP. A&B) CD-D594G and wild-type (WT) CD-BRAF were incubated with ATP (0, 0.475, 4.75, 47.5, 475, 950, 1900, and 3800 µM) and 10 mM Mg^{2+} then analyzed by DSF. The ΔT_m values are shown in a table to the right of each graph indicating the T_m at each concentration of ATP.

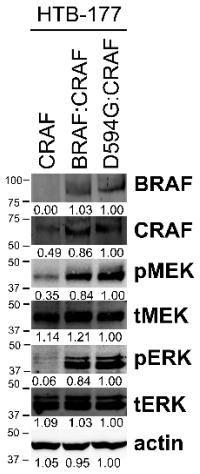


Figure S2: Heterodimers Activate the MAPK Pathway in Lung Cancer. CRAF, BRAF:CRAF, and D594G:CRAF were transiently transfected into HTB-177 lung cancer cells harboring KRAS^{Q61H}. The lysates were probed for the indicated protein using immunoblotting. Relative intensities were calculated using image J. The molecular weight marker sizes are shown to the left of the blots.

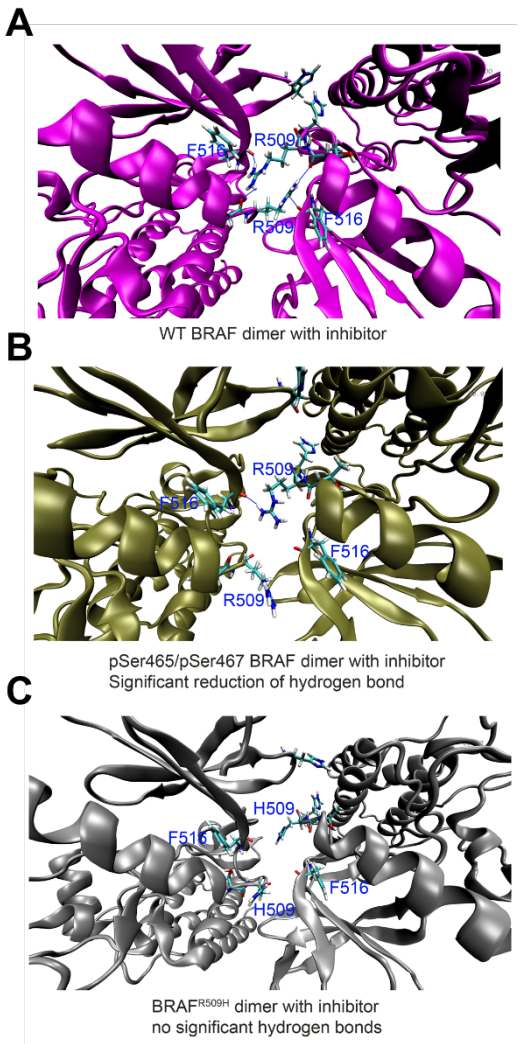


Figure S3: Evaluation of the Dimer Interfaces of BRAF Variants. A) WT BRAF dimer bound with ATP-competitive inhibitor (top). **B)** P-loop phosphorylated (pSer465/pSer467) BRAF dimer bound with ATP-competitive inhibitor (middle). **C)** $BRAF^{R509H}$ dimer bound with ATP-competitive inhibitor.

A

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BRAF 463  I GSGSFGTV
NEK11 35  LGSGSFGTV
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B

NEK11	TAO Kinase 2	GSK3B
EGFR	CK1D	ROSI
HER2	CK1E	YANK1
HER3	PAS	ACK
PIM 1	MEK 1	RSK2
PI3K	TGF1 BETA RECEPTOR	NIK
TNNI3k	SUGEN (SgK494)	IGF1R
STK33	TBK1	WNK1
Protein kinase A	ERBB4	STK24 (MST3)
Aurora B	SYK	PTK6
tyrosine-protein kinase Tec	WEE1	PDK-1
PFTAIRE	NEK1	MST4
RIP	ITK	FLT3
ROS Precursor	PRKCD	leucine-rich repeat serine/threonine-protein kinase 2
BMX	PIM3	

Figure S4: The Serine Residue within the P-Loop is Highly Conserved among Protein Kinases. A) Sequence alignment of two serine/threonine protein kinases, BRAF and NEK11. Complementary amino acid sequences are shown in black, while differences are highlighted in red. **B)** Kinase families that have one or two conserved P-loop (GxGxxG) serine residues are shown.